Interfacial Protein-Lipid Interactions I

2760-Pos Board B746
Human Dystrophin Rod 11-15 Sub-Domain: A Membrane Interacting Zone Modulated by Lipid Packing
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Dystrophin is essential for skeletal muscle function and confers resistance to the sarcolemma by interacting with cytoskeletal and membrane partners. We investigated here protein-lipid interaction of a five repeat from the central domain of dystrophin (DYS R11-15) also referred as an actin binding domain. In a first step, we demonstrated that DYS R11-15 interacts more strongly with anionic than with zwitterionic small unilamellar vesicles. Using large unilamellar vesicles with different radius and trypsin accessibility assays, we showed that the protein presents different conformation depending on vesicle curvature and lipid nature. Using label-free quantification mass spectroscometry, a protein domain protected from proteolysis in presence of anionic vesicles was observed while a protein domain more accessible to trypsin in the presence of either anionic or zwitterionic vesicles was identified. In a second step, we studied the adsorption behavior of the protein at the air-liquid and lipid-liquid interface in a Langmuir trough. DYS R11-15 displayed a surface activity while maintaining its α-helical secondary structure as shown by PM-IRRAS. At 16mN/m lateral pressure of monolayer lipid film, few protein clusters were observed by AFM, while at 20 and 30mN/m, a striking protein network was formed with both negative and zwitterionic phospholipids. However, image analysis and behaviour of the networks towards trypsination in the trough as revealed by AFM showed that trypsin accessibility to the protein network depends on the surface pressure as well as on the nature of the phospholipid. These results indicate that DYS R11-15 constitutes part of the dystrophin protein for which anchoring and interaction with membrane depend on the packing and the nature of lipids. Such behaviour provides a strong experimental support for a physiological role of dystrophin central domain in contraction-relaxation cycles and dynamics of muscle cells.

2761-Pos Board B747
Membrane Interactions of Apolipoprotein C-III Probed by Tryptophan Fluorescence
Robert L. Walker, Jennifer C. Lee.
Understanding how human apolipoproteins (apoPs) interact with cellular membranes is important because they are involved in lipid metabolism and their dysfunction can have detrimental health consequences. Specifically, studies of their conformational rearrangements in the presence of lipids are needed since apoPs have been found as aggregated fibrillar forms (amyloid) in atherosclerotic plaques. In our efforts to investigate the role of lipids in amyloid formation, we first sought to determine the membrane interactions of apolipoprotein C-III (apo C-III) using synthetic phospholipid vesicles as membrane mimics. Single tryptophan probes (W42, W54, and W65) will be prepared by site-directed mutagenesis with replacement of two of the three native Trp with Phe residues. Circular dichroism spectroscopy will be employed to monitor secondary structure formation of wild-type apo C-III and its single Trp mutants upon bilayer association, and equilibrium binding curves will be evaluated. Site-specific polypeptide-membrane interactions will be determined by steady-state and time-resolved tryptophan fluorescence measurements.

2762-Pos Board B748
Evaluation of Surfactant Inhibition and Reactivation by Captive Bubble Surfactometry
Lopez Rodriguez Elena, Olga Lucia Ospina Ramirez, Mercedes Echaide, H Williams Taeusch, Jesus Perez Gil.
Pulmonary surfactant is a complex mixture of lipids and proteins, which forms a film at the air-water interface in the alveoli. Its main function is to lower the surface tension at this interface thus decreasing the work of breathing and preventing alveolar collapse. Dysfunction of surfactant in the lungs is associated with respiratory distress syndromes such as acute respiratory distress syndrome (ARDS) or meconium aspiration syndrome (MAS). Many substances have been described as inhibitory agents for pulmonary surfactant interfacial activity. Three of the most common *in vivo* are serum, cholesterol and meconium. The behaviour of surfactant films at the interface can be followed under physiologically relevant conditions in the Captive Bubble Surfactometer (CBS). This includes i) examination of rapid film formation by adsorption of surfactant into the interface of an air bubble, ii) monitoring re-adsorption of material from surface-associated reservoirs into the interface of the bubble upon expansion or iii) analysis of film dynamic properties during compression-expansion cycling of the interface. Using the CBS with a modified protocol that allows exposure of surfactant to high concentrations of inhibitory compounds, we have evaluated the effect of several agents on the biophysical properties of lung surfactant films under physiologically meaningful constraints. In this model assay, we observe significant differences in the interfacial performance of surfactant preparations in the presence or absence of inhibitory agents such as serum or meconium, which determine the importance of surfactant composition and structure on its susceptibility to inactivation. Moreover we have confirmed the higher resistance to inhibition of complexes of pulmonary surfactant with ionic and non-ionic polymers and the potential of these additives to design new therapeutic surfactant preparations.

2763-Pos Board B749
Uv-Visible Spectroscopy of Glycoproteins Interacting with Lipid Vesicles
Andrew K. Fraser, Kwame K.E. Newon, Bruce D. Ray, Horia I. Petrache.
Glycoproteins are a class of proteins that are decorated with sugar (glycan) chains. Most known glycoproteins have been shown to play a role in intercellular interactions but the exact role of the glycan chains is still under investigation. Here we use light spectroscopy to study the interaction of three glycoforms of the protein Ovalbumin (A1, A2, and A3) with model membranes. These proteins are obtained from hen egg white. Phenylalanine, tryptophan, and tyrosine amino acids absorb ultraviolet light and each residue has a characteristic spectrum. When a protein interacts with a lipid membrane its absorbance spectrum is altered due to changes in the environment. This alteration is evidenced by shifts in the location of characteristic peaks in the second derivative spectrum. Measuring the shifts caused by the addition of lipid allows us to describe the interaction of glycosylated proteins with lipid membranes. By comparing peak shifts for different glycoforms against each other and against standard peptides provides insight into the role of glycans in cell membranes interactions.

2764-Pos Board B750
The Interactions of Viral Matrix Proteins with Membranes
James A. Freeth, John Sanderson, Paul Yeo.
Matrix proteins form the main structure of an enveloped virus, connecting the nucleocapsid to the phospholipid membrane. We have studied the M1 proteins from HRSV and Influenza A to determine their structural characteristics and the nature of their lipid binding. We have done this using Langmuir-Blodgett trough methods to study binding of matrix proteins to different lipid systems; and fluorescent confocal microscopy and FRET studies using giant unilamellar vesicles to simulate the proteins interactions at the cell plasma membrane.

2765-Pos Board B751
Charged Residues that Control the Electrostatic Association of Synaptotagmin 1 C2AB with Membranes
Anusa Thapa, Wei-wei Kuo, David Cafiso.
Signaling in the central nervous system depends on the fusion of synaptic vesicles to the presynaptic membrane of the neuron. This fusion event is mediated by membrane-anchored SNAREs (Soluble NSF Attachment Protein Receptors), but requires a number of additional protein factors including munc18, complexin and Synaptotagmin 1 (Syt1). Synaptotagmin 1 has been shown to act as the Ca^{2+} sensor in neuronal fusion, and it could act by bridging bilayers and assisting SNARE-mediated fusion, or it might directly interact with the SNAREs to assist SNARE assembly. Recent work has indicated that two arginine residues (R398 and R399) on the face opposite the calcium binding region in C2B domain are critical for the function of Syt1 in neurotransmitter release (Xue et al. (2008), NSMB 15, 1160) and it has been proposed that these residues function in associating this region to the membrane interface. Using site-directed spin labeling and progressive power saturation, we have shown that in a soluble fragment of Syt1 containing the two C2 domains (C2AB), that the region opposite the Ca^{2+}-binding sites in the C2B domain associates with the membrane interface and allows Syt1 to bridge two bilayers. When the single mutation R398Q and the double mutation R398Q R399Q are made to C2AB, power saturation measurements indicate that this region no longer associates with the membrane interface. Work is currently underway to test the role of these arginine residues in SNARE association. This work was supported by a grant from NIGMS, GM 072694.